Supplementary information for "Plasticity-led evolution: evaluating the key prediction of frequency-dependent adaptation"

Main text citation

Levis N.A., Pfennig D.W. 2019 Plasticity-led evolution: evaluating the key prediction of frequency-dependent adaptation. *Proceedings of the Royal Society of London B*. doi: 10.1098/rspb.2018.2754.

Supplementary methods for Section 2b: Testing whether frequency of trait expression predicts its adaptive refinement

Below, we describe the biological interpretation of the models that were compared using likelihood ratio tests. If there is no diet-dependent growth and no difference between selective environments, then the null model should be the best fit. In contrast, if diet alone is the best model, then detritus- and shrimp-fed tadpoles have differences in growth, but there have been no evolved changes in growth on a particular diet between selective environments. If any of the remaining models is deemed the best, then this would indicate an evolved difference between selective environments. Specifically, if selective environment alone is the best predictor, then this would indicate that overall growth is different between selective environments, but there is no diet-dependent effect on growth. The additive model (containing both diet and selective environment as fixed effects) being the best would show that there is parallel, diet-dependent growth in both selective environments, but that the overall magnitude of growth in the derived environment (sympatry) has evolved to be greater or less than in the ancestral environment (allopatry). Finally, if the model containing the interaction between diet and selective

environment is the best, then this would indicate non-parallel response to diet between selective environments that may or may not have differences in elevation. Essentially, this is the catch-all model for any evolved change in the direction and magnitude of growth. Importantly, if our prediction is supported, then the interaction model should be the best with sympatric tadpoles having higher growth on shrimp and lower growth on detritus than allopatric tadpoles.

Supplementary methods for Section 2c: Evaluating mechanisms of adaptive refinement

Based on previous work on this system [1-9], we explored five non-mutually exclusive mechanisms that might underlie these observed differences in competitive ability between selective environments. We specifically tested whether tadpoles from the two selective environments have diverged in: 1) intrinsic growth rate, 2) time budgets, 3) trait integration, 4) shrimp capture ability, or 5) trophic morphology.

First, partly based on Pfennig and Pfennig [9], we tested if there were differences in intrinsic growth rate between selective environments on either diet. To do so, we compared the growth of singleton tadpoles from the two selective environments [described in Section (b) above]. We compared models using likelihood ratio test as with the competition experiment. We only included 'sibship' and 'replicate' as random effects (i.e., the sibship identity of the competing family was not included).

Second, partly based on Pfennig and Murphy [4], we tested if there were differences in how much time tadpoles spent foraging. To do so, we observed 10 tadpoles from nine allopatric and six sympatric families that had been reared for 12 days under identical conditions on a detritus diet in the laboratory. Following Pfennig and Murphy [4], we lined the bottom a clear aquarium (20 × 13.5 × 13.5 cm) with 1 mm of detritus and observed how much time an individual spent

swimming, resting, eating, and active (swimming + eating). Tadpoles were given five minutes to acclimate to the aquarium before a five-minute focal observation period. For the proportion of time doing each activity, we compared a null model only containing the random effect 'sibship' with a full model that retained this random effect and also included 'selective environment' (i.e., allopatry or sympatry) as a fixed effect. For these tests, we arcsine square-root transformed the data prior to analysis.

Third, partly based on Pfennig and colleagues [8], we tested if there were differences between selective environments in trait integration; i.e., the nature of the correlations among different component traits of the carnivore ecomorph (previous work on this system had suggested that trait integration can serve as a measure of the quality of carnivore phenotypes produced [1, 8]). To do so, we calculated the correlations among the morphological traits measured above for every combination of diets and rearing conditions (singleton or competition) in allopatric and sympatric tadpoles. We then performed the Fisher r-to-z transformation and compared the magnitude of the correlations using the function 'paired.r' in the 'psych' package of R. P values were corrected using the false discovery rate [10] with the 'qvalue' function in the qvalue package of R [11].

Fourth, partly based on Pfennig and colleagues [7], we tested if tadpoles from the two selective environments had diverged in their ability to capture shrimp; i.e., the time tadpoles took them to eat a standard quantity of live shimp (this previous work also suggested that shrimp eating time can serve as a measure of the *quality* of carnivore phenotypes produced [7]). To do so, we placed five tadpoles from nine allopatric and five sympatric sibships individually into opaque 3oz Dixie cups. We then gave each tadpole three adult brine shrimp and recorded the amount of time it took an individual to consume all three shrimp. We continuously observed

tadpoles for the first 60 minutes and then checked the cups every 15 minutes for the next five hours. Individuals that did not finish their shrimp were assigned the conservative estimate of 360 minutes. Our response variables was time to eat all shrimp. We compared a null model only containing the random effect 'sibship' with a full model that retained this random effect and also included 'selective environment' (i.e., allopatry or sympatry) as a fixed effect. In this case, models were fit with a Poisson distribution. We also determined if variation in shrimp capture time differed between the two selective environments using a Levene's test. If refinement has occurred, we would expect lower variation for sympatric tadpoles compared to that of allopatric tadpoles.

Finally, partly based on Pfennig and Murphy [4] and Pfennig and colleagues [7], we tested if there were differences in trophic morphology between selective environment. To do so, we measured the following four morphological traits that are diagnostic of morphotype [2, 3, 7]: the width of the jaw muscle (orbitohyoideus muscle; OH), the number of denticle rows (DR), the number of gut coils (GC), and the shape of the mouthparts (MP). We standardized OH for body size (SVL) by regressing log OH on log SVL [6, 7]. In *Spea*, more carnivore-like tadpoles tend to have a large OH, few DR, few GC, and highly keratinized MP [2, 3, 7]. For each variable, we then performed a type III ANOVA on a full model containing the fixed terms 'diet', 'selective environment', 'treatment type' and all possible interactions, and the random terms 'replicate' and 'sibship'. MP, DR, and GC were all analyzed using a Poisson distribution. If significant, we further explored interaction terms using type III sum of squares ANOVAs and post hoc multiple comparisons tests on fixed effects. The latter were performed using the 'pairwise.t.test' function with 'fdr' correction in R.

This last analysis also tested for evolved differences in phenotypic plasticity between selective environments. Given that tadpoles in sympatry less frequently exhibit both alternative morphs, we predicted that these tadpoles would have reduced diet-dependent plasticity compared to those from allopatry. If there is no diet-dependent plasticity in a trait and there has been no evolution between selective environments, then none of the fixed effects will be significant. In contrast, if diet is significant, then detritus- and shrimp-fed tadpoles have different levels of trait expression (i.e., there is diet-dependent plasticity), but there have been no evolved changes in plasticity between selective environments. If 'selective environment' is significant then this would indicate an evolved shift in trait production owing to selection—i.e., genetic accommodation has occurred [sensu 12]. Specifically, if selective environment is significant, then this would indicate that overall magnitude of trait expression is different between selective environments, but there is no diet-dependent plasticity. If both diet and selective environment are significant then there is parallel, diet-dependent plasticity in both selective environments, but that the overall magnitude of trait expression in the derived environment (sympatry) has evolved to be greater or less than in the ancestral environment (allopatry). Finally, if the interaction between diet and selective environment is significant, then this would indicate non-parallel reaction norms between selective environments that may or may not have differences in elevation. This model represents any evolved change in the direction and magnitude of trait plasticity. As one example, if tadpoles from the allopatric (ancestral) selective environment show diet-dependent plasticity in a trait, but tadpoles from the sympatric (derived) selective environment do not, then this would indicate evolution by genetic assimilation for that trait. Note, however, that genetic assimilation is only one of several alternative patterns of evolved plastic responses that are possible [13].

Table S1. Collection information for pairs used in these experiments.

Pair (number)	Sex	Latitude	Longitude	Selective Environment
A (1)	F	34.6402	-99.3340	Allopatry
A (1)	M	34.6402	-99.3340	Allopatry
B (2)	F	40.0466	-101.5357	Allopatry
B (2)	M	40.0466	-101.5357	Allopatry
C (4)	F	39.6782	-104.0415	Allopatry
C (4)	M	39.6782	-104.0415	Allopatry
D (7)	F	40.0850	-101.4741	Allopatry
D (7)	M	40.0850	-101.4741	Allopatry
E (8)	F	41.9833	-100.3116	Allopatry
E (8)	M	41.9833	-100.3116	Allopatry
F (10)	F	33.9157	-98.4901	Allopatry
F (10)	M	33.9157	-98.4901	Allopatry
G (11)	F	34.6402	-99.3340	Allopatry
G (11)	M	39.3065	-102.2692	Allopatry
H (12)	F	39.3871	-102.5939	Allopatry
H (12)	M	39.7412	-103.5932	Allopatry
I (3)	F	39.7337	- 103.8647	Allopatry
I (3)	M	39.7337	- 103.8647	Allopatry
J (6)	F	39.7589	-103.5178	Allopatry
J (6)	M	39.7543	-104.0412	Allopatry
K (14)	F	31.7397	-109.0988	Sympatry
K (14)	M	31.7397	-109.0988	Sympatry
L (16)	F	31.7397	-109.0988	Sympatry
L (16)	M	31.7397	-109.0988	Sympatry
M (19)	F	31.8354	-109.0321	Sympatry
M (19)	M	31.7882	-109.0641	Sympatry
N (21)	F	31.7882	-109.0641	Sympatry
N (21)	M	31.8124	-109.0518	Sympatry
O (22)	F	31.7408	-109.0767	Sympatry
O (22)	M	31.7397	-109.0988	Sympatry
P (23)	F	31.7364	-109.1008	Sympatry
P (23)	M	31.8124	-109.0518	Sympatry
Q (15)	F	31.7397	-109.0988	Sympatry
Q (15)	M	31.7397	-109.0988	Sympatry
R (18)	F	31.7882	-109.0641	Sympatry
R (18)	M	31.7882	-109.0641	Sympatry

The number in parentheses corresponds to the family identity number associated with the data accompanying this work.

Table S2. Summary statistics comparing intrinsic growth rate between sympatric and allopatric tadpoles reared on alternative diets. P values less than 0.05 indicate that a given model was significantly better than the one above it. Diet was the only significant predictor of intrinsic growth rate.

Model	AIC	logLike	χ^2	*P*
Null	793.25	-392.62	---	---
Diet	768.54	-379.27	26.70	2.4×10^{-7}
Selective environment	795.24	-392.62	0.00	1.000
Diet + Selective environment	770.54	-379.27	26.70	2.4×10^{-7}
Diet:Selective environment	772.28	-379.14	0.26	0.613

Table S3. Results from behavioral time budget assay comparison between tadpoles whose parents were derived allopatry versus sympatry. Time spent performing the behaviors did not differ between selective environments.

Resting	AIC	logLike	χ^2	*P*
Null	106.49	-50.25	---	---
Selective environment	107.96	-49.98	0.53	0.466
Eating	AIC	logLike	χ^2	*P*
Null	-57.30	31.65	---	---
Selective environment	-55.73	31.87	0.43	0.510
Swimming	AIC	logLike	χ^2	*P*
Null	97.85	-45.93	---	---
Selective environment	99.51	-45.76	0.34	0.559
Active	AIC	logLike	χ^2	*P*
Null	104.56	-49.28	---	---
Selective environment	106.18	-49.09	0.38	0.536

Table S4. Summary of results from comparison of trait integration between tadpoles whose parents were derived from sympatry or allopatry.

Test	Diet	Rearing	Trait.1	Trait.2	Allopatry Correlation	Sympatry Correlation	Z	p	q
1	Detritus	Competition	MP	DR	-0.070	0.163	1.15	0.250	0.863
2	Shrimp	Competition	MP	DR	0.082	0.074	0.04	0.970	0.957
3	Combined	Competition	MP	DR	0.001	0.131	0.92	0.360	0.875
4	Detritus	Singleton	MP	DR	0.210	0.114	0.48	0.630	0.932
5	Shrimp	Singleton	MP	DR	-0.031	-0.136	0.52	0.600	0.932
6	Combined	Singleton	MP	DR	0.110	-0.032	1.00	0.320	0.875
7	Detritus	Competition	DR	GC	0.100	0.290	0.97	0.330	0.875
8	Shrimp	Competition	DR	GC	0.265	0.128	0.70	0.480	0.883
9	Combined	Competition	DR	GC	0.170	0.243	0.53	0.590	0.932
10	Detritus	Competition	MP	GC	-0.015	-0.157	0.70	0.480	0.883
11	Shrimp	Competition	MP	GC	0.178	-0.099	1.37	0.170	0.829
12	Combined	Competition	MP	GC	0.064	-0.083	1.04	0.300	0.875
13	Detritus	Singleton	DR	GC	0.144	0.284	0.72	0.470	0.883
14	Shrimp	Singleton	DR	GC	0.189	-0.002	0.94	0.340	0.875
15	Combined	Singleton	DR	GC	0.142	0.159	0.13	0.900	0.953
16	Detritus	Singleton	MP	GC	0.058	0.028	0.15	0.880	0.953
17	Shrimp	Singleton	MP	GC	0.021	-0.124	0.72	0.470	0.883
18	Combined	Singleton	MP	GC	0.018	-0.014	0.22	0.820	0.953
19	Detritus	Competition	DR	OH	0.038	0.297	1.32	0.190	0.829
20	Shrimp	Competition	DR	OH	-0.079	0.181	1.29	0.200	0.829
21	Combined	Competition	DR	OH	-0.006	0.244	1.79	0.070	0.829
22	Detritus	Competition	GC	OH	0.101	0.233	0.69	0.490	0.883
23	Shrimp	Competition	GC	OH	-0.030	0.004	0.17	0.870	0.953
24	Combined	Competition	GC	OH	-0.040	0.155	1.38	0.170	0.829
25	Detritus	Competition	MP	OH	0.055	0.066	0.06	0.950	0.957
26	Shrimp	Competition	MP	OH	0.012	-0.040	0.26	0.800	0.953
27	Combined	Competition	MP	OH	0.038	0.021	0.12	0.900	0.953
28	Detritus	Singleton	DR	OH	0.048	-0.041	0.43	0.660	0.936
29	Shrimp	Singleton	DR	OH	-0.006	0.020	0.12	0.900	0.953
30	Combined	Singleton	DR	OH	0.027	-0.013	0.28	0.780	0.953
31	Detritus	Singleton	GC	OH	-0.258	0.027	1.40	0.160	0.829
32	Shrimp	Singleton	GC	OH	-0.263	-0.184	0.41	0.680	0.936
33	Combined	Singleton	GC	OH	-0.262	-0.047	1.56	0.120	0.829
34	Detritus	Singleton	MP	OH	-0.105	-0.002	0.51	0.610	0.932
35	Shrimp	Singleton	MP	OH	0.251	0.359	0.59	0.560	0.932

36	Combined	Singleton	MP	OH	0.084	0.303	1.61	0.110	0.829
37	Combined	Both	MP	DR	0.043	0.053	0.10	0.920	0.953
38	Combined	Both	MP	GC	0.039	-0.081	1.20	0.230	0.863
39	Combined	Both	MP	OH	0.057	0.192	1.38	0.170	0.829
40	Combined	Both	DR	GC	0.161	0.199	0.39	0.700	0.936
41	Combined	Both	DR	OH	0.010	0.097	0.87	0.380	0.875
42	Combined	Both	GC	OH	-0.123	0.026	1.50	0.130	0.829

Table S5. Results of type III sum of squares ANOVA on trophic traits. For each trait, bolded values denote which variables were significant predictors.

	Mouthpart score		Number of denticle rows		Orbitohyoideus width		Number of gut coils	
Term	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*
Intercept	**22.18**	**2.48×10^{-6}**	**111.00**	**2.00×10^{-16}**	**8.67**	**0.003**	**1029.53**	**2.00×10^{-16}**
Diet	0.01	0.939	0.31	0.576	**8.85**	**0.003**	0.53	0.465
Selective environment	**8.21**	**0.004**	**9.59**	**0.002**	**7.58**	**0.006**	0.25	0.615
Treatment type	0.14	0.705	1.55	0.214	0.42	0.518	0.29	0.593
Diet:Selective environment	0.22	0.635	0.00	0.977	**4.49**	**0.034**	1.66	0.198
Diet:Treatment type	0.12	0.728	0.08	0.776	1.69	0.194	2.29	0.130
Selective environment: Treatment type	0.04	0.836	2.63	0.105	0.25	0.620	1.42	0.233
Diet:Selective environment:Treatment type	0.02	0.887	0.94	0.333	**6.52**	**0.011**	0.17	0.6776

Table S6. (A) Summary of type III sum of squares ANOVA of orbitohyoideus (OH) width from tadpoles reared in competition. (B) Results from post-hoc comparison among selective environment-diet groups. Values denote the distance between group means in OH width and the associated P value in parentheses. In both panels A and B, bolded values indicate statistical significance.

A. Type III sum of squares ANOVA			
Term	χ^2	*P*	
Intercept	**8.37**	**0.004**	
Diet	**9.06**	**0.003**	
Selective environment	**6.91**	**0.009**	
Diet:Selective environment	**5.09**	**0.024**	
B. Multiple comparisons test			
Group	Allo.det	Allo.shr.	Sym.det
Allo.shr	**0.048 (0.018)**	---	---
Sym.det	**0.061 (0.003)**	0.013 (0.578)	---
Sym.shr	**0.067 (0.002)**	0.019 (0.446)	0.006 (0.733)

Table S7. Summary of type III sum of squares ANOVA of orbitohyoideus (OH) width from tadpoles reared as singletons. Bolded values indicate statistical significance.

Term	χ^2	*P*
Intercept	**6.11**	**0.013**
Diet	1.41	0.236
Selective environment	**6.27**	**0.012**
Diet:Selective environment	3.18	0.075

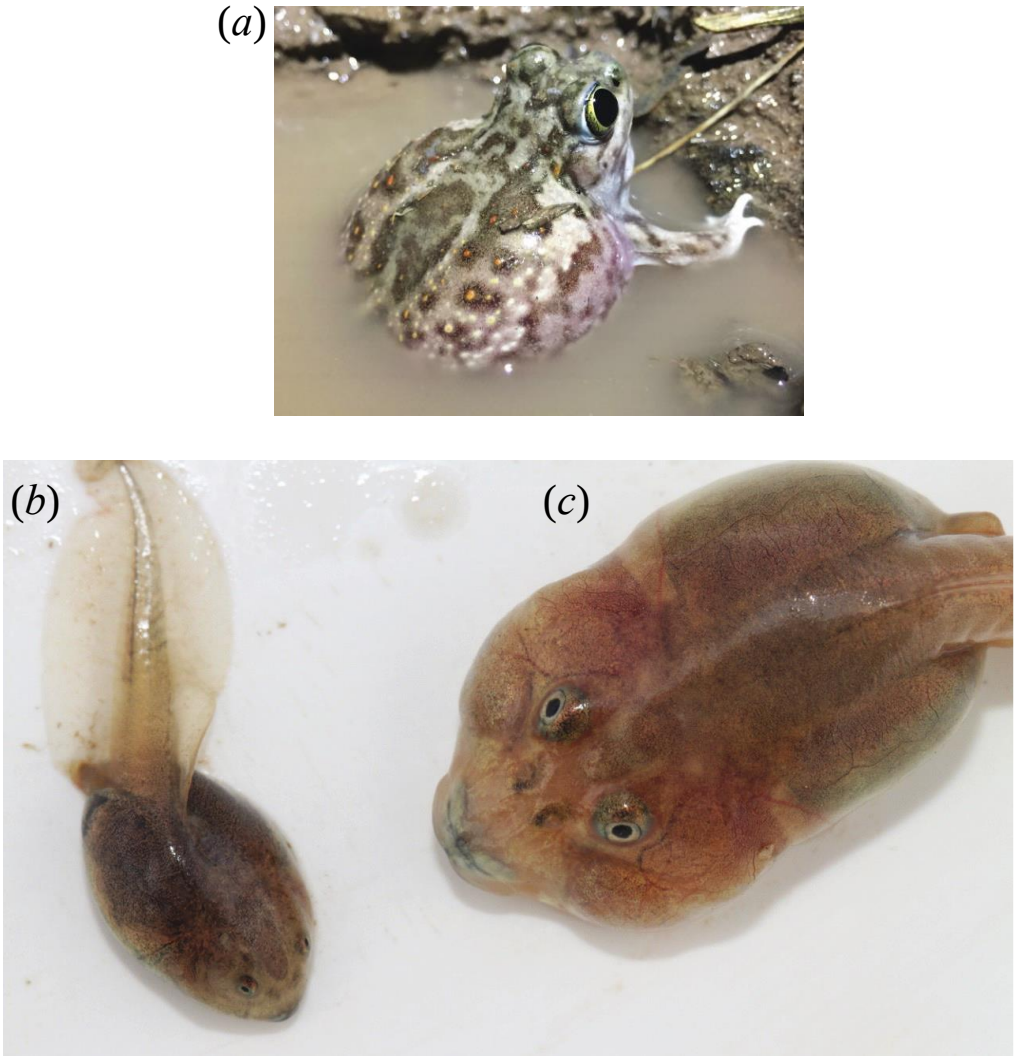

Figure S1. (a) In many populations, plains spadefoot toads, *Spea bombifrons*, produce among their tadpoles either (b) omnivores or (c) carnivores. These individuals were photographed in the wild in the San Simon Valley of Arizona (Photos by D. Pfennig).

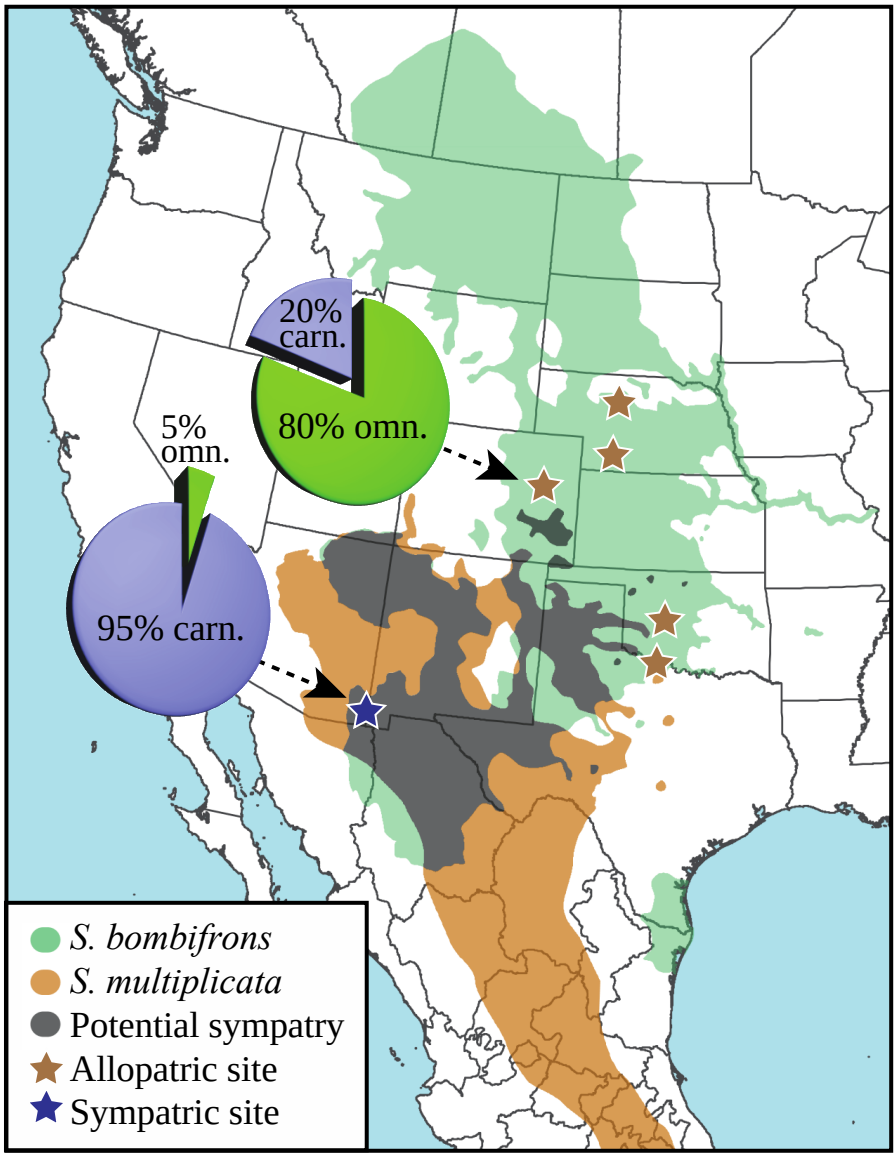

Figure S2. Geographical ranges of *Spea bombifrons* and *S. multiplicata*, showing: 1) locations of collection sites for adults used to generate tadpoles for the experiments (stars), and 2) previously estimated percentages of each ecomorph produced in the wild within each selective environment (pie charts). See main text for details.

Appendix 1. Calculation of carnivore subpopulation size in allopatry and sympatry

1) We estimated the number of males attending allopatric breeding aggregations from observations made by Rebecca O'Brien (personal communication) of 17 ponds. We estimated the number of males attending breeding aggregations at our focal sympatric ponds from personal observations (D. Pfennig) and personal communication with Karin Pfennig.

2) We estimated the sex ratios of males and females in these two environments based on 20+ years of personal observations by D. and K. Pfennig and then estimated the number of breeding males based on this ratio.

3) We assumed each mated male produced 1000 tadpoles [9] and used data on morph frequencies in allopatry and sympatry from previous work [6] to estimate the number of carnivore tadpoles. We used the highest value for number of males in allopatry and lowest value for number of males in sympatry in order to have a conservative estimate of differences between selective environments.

4) Using these data and assumptions, we estimated the relative effect of drift on the carnivore subpopulation in allopatry compared to that in sympatry based on the relative proportion of carnivores produced in each environment.

Selective environment	Allopatry	Sympatry
Median # males	25	10
Sex ratio (F:M)	2:1	1:1
Breeding males	13	10
Assumed # tadpoles per bred male	1000	1000
Proportion carnivores	33% (upper limit from Pfennig et al. 2006)	86% (lower limit from Pfennig et al. 2006)
Number of carnivores	0.33 * 13000 = 4290	0.86 * 10000 = 8600

Based on the above, the number of carnivores in sympatry is at least 2 times (8600/4290) the number of carnivores in allopatry. Thus, drift on the carnivore subpopulation is 2x stronger in allopatry and selection would need to be twice as strong in allopatry to have a similar selection-drift influence as that seen in sympatry.

Appendix 2. Estimates of trait evolution in allopatry and sympatry

1) We combined field data on SVL, OH, and MP from previous studies [7, 14]. For OH, we only included measurements on the right OH muscle.

2) We calculated a morphological index (MI) by performing a principal component analysis of MP and residuals of ln(OH) regressed on ln(SVL) according to Pfennig et al. (2006) and determined the magnitude of difference in mean MI between selective environments.

3) We calculated the same MI for our singleton allopatric and sympatric tadpoles and determined the magnitude difference in mean MI between selective environments.

4) We calculated the covariance matrices between ln(SVL) (a proxy for fitness: ω) and MI (trait: z) for sympatry and allopatry separately.

5) We used the Price equation: $\Delta z = \text{cov}(\omega,z)$ and assumed perfect, equivalent heritabilities. However, it is likely that heritability of MI is greater in sympatry than in allopatry given the persistent presence of *S. multiplicata*. Therefore, this assumption likely underestimates differences in trait evolution between selective environments.

The difference in MI between our experimental allopatric and sympatric tadpoles was 1.29165 (sympatric larger than allopatric). In wild-caught tadpoles, the difference in MI was 1.41916 (again, sympatric was larger than allopatric). Previous data from wild-caught tadpoles suggests that the covariance between fitness [ln(SVL)] and phenotype (MI) was ~2x greater in sympatry

(0.04013) than allopatry (0.02103). Assuming allopatry and sympatry started at the same MI value, but experienced phenotype changes at different rates according to the Price equation, it would take ~68 generations to reach the magnitude of difference in MI observed for our singleton tadpoles and it would take ~75 generations to reach the magnitude of difference in MI observed among wild-caught tadpoles.

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